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Paper Alert

A selection of interesting papers that were recently published in major journals most likely to report significant results in structural biology, protein, and RNA folding.

☐ **Mechanism of glutamate receptor desensitization.** Yu Sun, Rich Olson, Michelle Horning, Neali Armstrong, Mark Mayer, and Eric Gouaux (2002). Nature *417*, 245–253.

Ligand-gated ion channels transduce chemical signals into electrical impulses by opening a transmembrane pore in response to binding one or more neurotransmitter molecules. After activation, many ligand-gated ion channels enter a desensitized state in which the neurotransmitter remains bound but the ion channel is closed. Using the GluR2 AMPA-sensitive glutamate receptor, the authors show that the ligand binding cores form dimers and that stabilization of the intradimer interface by either mutations or allosteric modulators reduces desensitization. Receptor activation involves conformational changes within each subunit that result in an increase in the separation of portions of the receptor that are linked to the ion channel. Desensitization occurs through rearrangement of the dimer interface, which disengages the agonist-induced conformational change in the ligand binding core from the ion channel gate.

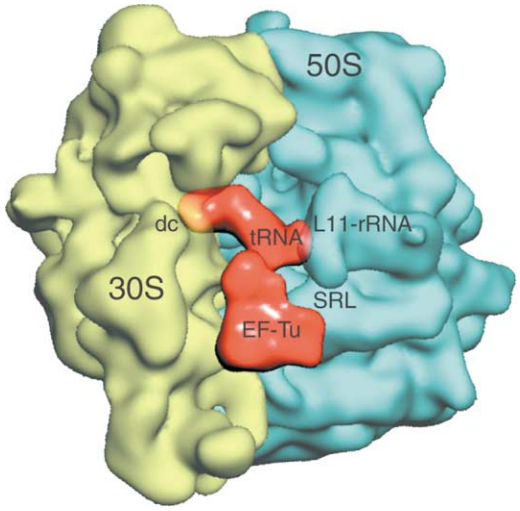
☐ **Rotavirus protein involved in genome replication and packaging exhibits a HIT-like fold.** Hariharan Jayaram, Zenobia Taraporewala, John T. Patton, and B.V. Venkataram Prasad (2002). Nature *417*, 311–315.

Rotavirus, the major cause of life-threatening infantile gastroenteritis, is a member of the *Reoviridae*. The nonstructural protein NSP2 of rotavirus, which exhibits nucleoside triphosphatase, single-stranded RNA binding, and nucleic-acid helix-destabilizing activities, is a major component of viral replicase complexes. The authors describe the crystal structure of the functional octamer of NSP2. The monomer has two distinct domains. The amino-terminal domain has a new fold. The carboxy-terminal domain resembles the histidine triad (HIT) group of nucleotidyl hydrolases. This structural similarity suggests that the nucleotide binding site is located inside the cleft between the two domains. Prominent grooves that run diagonally across the doughnut-shaped octamer are probable locations for RNA binding. Several RNA binding sites, resulting from the quaternary organization of NSP2 monomers, may be required for the helix destabilizing activity of NSP2 and its function during genome replication and packaging.

☐ **The catalytic pathway of horseradish peroxidase at high resolution.** Gunnar I. Berglund, Gunilla H. Carlsson, Andrew T. Smith, Hanna Szöke, Anette Henriksen, and Janos Hajdu (2002). Nature *417*, 463–468.

A molecular description of oxygen and peroxide activation in biological systems is difficult, because electrons liberated during X-ray data collection reduce the active centers of redox enzymes catalyzing these reactions. Here the authors describe an effective strategy to obtain crystal structures for high-valency redox intermediates and present a three-dimensional movie of the X-ray-driven catalytic reduction of a bound dioxygen species in horseradish peroxidase (HRP). High-resolution structures could be obtained for all five oxidation states of HRP, showing such structures with preserved redox states for the first time.

☐ **Cryo-EM reveals an active role for aminoacyl-tRNA in the accommodation process.** Mikel Valle, Jayati Sengupta, Neil K. Swami, Robert A. Grassucci, Nils Burkhardt, Knud H. Nierhaus, Rajendra K. Agrawal, and Joachim Frank (2002). EMBO J. *21*, 3557–3567.



The authors have used cEM and image reconstruction of a blocked ribosome to demonstrate that the monitoring of a conformational change in the tRNA is a key part of the decoding mechanism that imparts the exquisitely accuracy to translation. The monitoring of this change involved dealing with heterogeneity in the population so that the fraction of the ribosomes with full occupancy could be reconstructed. The figure shows a cEM map of the EF-Tu•tRNA•GDP ternary complex bound to the 70S ribosome from *E. coli*. The 3D reconstruction was calculated with a sample containing the antibiotic kirromycin, which blocks the conformational changes of EF-Tu and stalls the ternary complex while the aminoacyl-tRNA is being delivered to the ribosomal A site. The ribosomal subunits are shown semitransparent (50S blue and 30S yellow). In the density attributable to the ternary complex (red), tRNA and EF-Tu interact with both ribosomal subunits. The aminoacyl-tRNA reaches the decoding center (dc). The codon-anticodon pairing is evidently facilitated by a kink in the anticodon arm of

the tRNA. The GTP hydrolysis by EF-Tu is controlled by a network of interactions between tRNA, EF-Tu, and the regions of the 50S subunit involved in ribosomal factor binding (L11-rRNA and SRL). Labeling: dc, decoding center; L11-rRNA, protein L11 and rRNA complex; SRL, sarcin ricin loop.

□ **Crystal structure and mechanism of a calcium-gated potassium channel.** Youxing Jiang, Alice Lee, Jiayun Chen, Martine Cadene, Brian T. Chait, and Roderick Mackinnon (2002). Nature *417*, 515–522.

Ion channels exhibit two essential biophysical properties; that is, selective ion conduction, and the ability to gate-open in response to an appropriate stimulus. Two general categories of ion channel gating are defined by the initiating stimulus: ligand binding (neurotransmitter- or second-messenger-gated channels) or membrane voltage (voltage-gated channels). The authors present the structural basis of ligand gating in a K^+ channel that opens in response to intracellular Ca^{2+}. They cloned, expressed, analyzed electrical properties, and determined the crystal structure of a K^+ channel (MthK) from *Methanobacterium thermoautotrophicum* in the Ca^{2+}-bound, opened state. Eight RCK domains (regulators of K^+ conductance) form a gating ring at the intracellular membrane surface. The gating ring uses the free energy of Ca^{2+} binding in a simple manner to perform mechanical work to open the pore. In an accompanying paper, the structure of the open pore conformation is described (Jiang et al. [2002], Nature *417*, 523–526).

□ **The complex of Arl2-GTP and PDE: from structure to function.** Michael Hanzal-Bayer, Louis Renault, Pietro Roversi, Alfred Wittinghofer, and Roman C. Hillig (2002). EMBO J. *21*, 2095–2106.

Arf-like (Arl) proteins are close relatives of the Arf regulators of vesicular transport, but their function is unknown. The authors present the crystal structure of full-length Arl2-GTP in complex with its effector PDE solved in two crystal forms. Arl2 shows a dramatic conformational change from the GDP-bound form, which suggests that it is reversibly membrane associated. PDE is structurally closely related to RhoGDI and contains a deep empty hydrophobic pocket. Further experiments show that H-Ras, Rheb, Rho6, and Gi1 interact with PDE and that, at least for H-Ras, the intact C terminus is required. PDE may be a specific soluble transport factor for certain prenylated proteins and Arl2-GTP a regulator of PDE-mediated transport.

□ **Aura virus structure suggests that the T = 4 organization is a fundamental property of viral structural proteins.** Wei Zhang, Bonnie R. Fisher, Norman H. Olson, James H. Strauss, Richard J. Kuhn, and T. S. Baker (2002). J. Virol. *76*, 7239–7246.

Aura and Sindbis viruses are closely related alphaviruses. Unlike other alphaviruses, Aura virus efficiently encapsidates both genomic RNA (11.8 kb) and subgenomic RNA (4.2 kb) to form virus particles. Previous studies on negatively stained Aura virus particles predicted that there were two major size classes with putative T = 3 and T = 4 capsid structures. If so, the fusion protein shells of these two form would have to change dramatically between these two forms. The authors combined cEM and three-dimensional image reconstruction techniques to examine the structures of the two different classes of Aura virus particles separated on sucrose gradients. Aura virus particles of both top and bottom components have similar, T = 4 structures that resemble those of other alphaviruses. Hence, the previously observed difference in size is a result of negative staining of the particles and disappears when the native structure is maintained in water by cEM.

□ **Crystal structure of the tetrameric Mad1-Mad2 core complex: implications of a 'safety belt' binding mechanism for the spindle checkpoint.** Lucia Sironi, Marina Mapelli, Stefan Knapp, Anna De Antoni, Kuan-Teh Jeang, and Andrea Musacchio (2002). EMBO J. *21*, 2496–2506.

The spindle checkpoint protein Mad1 recruits Mad2 to unattached kinetochores and is essential for Mad2-Cdc20 complex formation in vivo but not in vitro. The crystal structure of the Mad1-Mad2 complex reveals an asymmetric tetramer, with elongated Mad1 monomers parting from a coiled-coil to form two connected subcomplexes with Mad2. The Mad2 C-terminal tails are hinged mobile elements wrapping around the elongated ligands like molecular 'safety belts'. The authors show that Mad1 is a competitive inhibitor of the Mad2-Cdc20 complex, and propose that the Mad1-Mad2 complex acts as a regulated gate to control Mad2 release for Cdc20 binding. Mad1-Mad2 is strongly stabilized in the tetramer, but a 1:1 Mad1-Mad2 complex slowly releases Mad2 for Cdc20 binding, driven by favorable binding energies. Thus, the rate of Mad2 binding to Cdc20 during checkpoint activation may be regulated by conformational changes that destabilize the tetrameric Mad1-Mad2 assembly to promote Mad2 release. They also show that unlocking the Mad2 C-terminal tail is required for ligand release from Mad2, and that the 'safety belt' mechanism may prolong the lifetime of Mad2-ligand complexes.

□ **Virus-like particles of a fish nodavirus display a capsid subunit domain organization different from that of insect nodaviruses.** Liang Tang, Chian-Shing Lin, Noel K. Krishna, Mark Yeager, Anette Schneemann, and J.E. Johnson (2002). J. Virol. *76*, 6370–6375.

The structure of recombinant virus-like particles of malabaricus grouper nervous necrosis virus (MGNNV), a fish nodavirus isolated from the grouper *Epinephelus malabaricus*, was determined by cEM and three-dimensional reconstruction at 23 Å resolution. The cEM structure, sequence comparison, and protein fold recognition analysis indicate that the coat protein of MGNNV has two domains resembling those of tomato bushy stunt

virus and Norwalk virus, rather than the expected single-domain coat protein of insect nodaviruses.

☐ **Structure of the globular tail of nuclear lamin.** Sirano Dhe-Paganon, Eric D. Werner, Young-In Chi, and Steven E. Shoelson (2002). J. Biol. Chem. *277*, 17381–17384.

The nuclear lamins form a two-dimensional matrix that provides integrity to the cell nucleus and participates in nuclear activities. Mutations in the region of human *LMNA* encoding the carboxyl-terminal tail Lamin A/C are associated with forms of muscular dystrophy and familial partial lipodystrophy (FPLD). To help discriminate tissue-specific phenotypes, the authors have solved the three-dimensional crystal structure of the lamin A/C globular tail at 1.4 Å. The domain adopts a novel, all immunoglobulin-like fold. FPLD-associated mutations cluster within a small surface, whereas muscular dystrophy-associated mutations are distributed throughout the protein core and on its surface. These findings distinguish myopathy- and lipodystrophy-associated mutations and provide a structural framework for further testing hypotheses concerning lamin function.

☐ **Structure of formamidopyrimidine-DNA glycosylase covalently complexed to DNA.** Rotem Gilboa, Dmitry O. Zharkov, Gali Golan, Andrea S. Fernandes, Sue Ellen Gerchman, Eileen Matz, Jadwiga H. Kycia, Arthur P. Grollman, and Gil Shoham (2002). J. Biol. Chem. *277*, 19811–19816.

Formamidopyrimidine-DNA glycosylase (Fpg) is a DNA repair enzyme that excises oxidized purines from damaged DNA. The Schiff base intermediate formed during this reaction between *Escherichia coli* Fpg and DNA was trapped by reduction with sodium borohydride, and the structure of the resulting covalently cross-linked complex was determined at a 2.1 Å resolution. Fpg is a bilobal protein with a wide, positively charged DNA binding groove. It possesses a conserved zinc finger and a helix-two turn-helix motif that participate in DNA binding. The absolutely conserved residues Lys-56, His-70, Asn-168, and Arg-258 form hydrogen bonds to the phosphodiester backbone of DNA, which is sharply kinked at the lesion site. Residues Met-73, Arg-109, and Phe-110 are inserted into the DNA helix, filling the void created by nucleotide eversion. A deep hydrophobic pocket in the active site is positioned to accommodate an inverted base. Structural analysis of the Fpg-DNA complex reveals essential features of damage recognition and the catalytic mechanism of Fpg.

☐ **The crystal structure of *Mycobacterium tuberculosis* alkylhydroperoxidase AhpD, a potential target for antitubercular drug design.** Christine M. Nunn, Snezana Djordjevic, Patrick J. Hillas, Clinton R. Nishida, and Paul R. Ortiz de Montellano (2002). J. Biol. Chem. *277*, 20033–20040.

The resistance of *Mycobacterium tuberculosis* to isoniazid is commonly linked to inactivation of a catalase-peroxidase, KatG, that converts isoniazid to its biologically active form. Loss of KatG is associated with elevated expression of the alkylhydroperoxidases AhpC and AhpD. AhpD has no sequence identity with AhpC or other proteins but has alkylhydroperoxidase activity and possibly additional physiological activities. The alkylhydroperoxidase activity, in the absence of KatG, provides an important antioxidant defense. The protein is a trimer in a symmetrical cloverleaf arrangement. Each subunit exhibits a new all-helical protein fold in which the two catalytic sulfhydryl groups, Cys-130 and Cys-133, are located near a central cavity in the trimer. The structure supports a mechanism for the alkylhydroperoxidase activity in which Cys-133 is deprotonated by a distant glutamic acid via the relay action of His-137 and a water molecule. The cysteine then reacts with the peroxide to give a sulfenic acid that subsequently forms a disulfide bond with Cys-130. The crystal structure of AhpD identifies a new protein fold relevant to members of this protein family in other organisms. The structural details constitute a potential platform for the design of inhibitors of potential utility as antitubercular agents and suggest that AhpD may have disulfide exchange properties of importance in other areas of *M. tuberculosis* biology.

☐ **Crystal structure of the Homer 1 family conserved region reveals the interaction between the EVH1 domain and own proline-rich motif.** Katsumasa Irie, Toru Nakatsu, Kaoru Mitsuoka, Atsuo Miyazawa, Kenji Sobue, Yoko Hiroaki, Tomoko Doi, Yoshinori Fujiyoshi, and Hiroaki Kato (2002). J. Mol. Biol. *318*, 1117–1126.

PSD-Zip45 (also named Homer 1c/Vesl-1L) is a synaptic scaffolding protein, which interacts with neurotransmitter receptors and other scaffolding proteins to target them into post-synaptic density (PSD), a specialized protein complex at the synaptic junction. Binding of the PSD-Zip45 to the receptors and scaffolding proteins results in colocalization and clustering of its binding partners in PSD. It has an Ena/VASP homology 1 (EVH1) domain in the N terminus for receptor binding, two leucine zipper motifs in the C terminus for clustering, and a linking region whose function is unclear despite the high level of conservation within the Homer 1 family. The crystallographic analysis of the largest fragment of residues 1–163, including an EVH1 domain reported here, demonstrates that the EVH1 domain contains an α-helix longer than that of the previous models, and that the linking part included in the conserved region of Homer 1 (CRH1) of the PSD-Zip45 interacts with the EVH1 domain of the neighbor CRH1 molecule in the crystal. The results suggest that the EVH1 domain recognizes the PPXXF motif found in the binding partners, and the SPLTP sequence (P-motif) in the linking region of the CRH1. The two types of binding are partly overlapped in the EVH1 domain, implying a mechanism to regulate multimerization of Homer 1 family proteins.

□ **Protein NMR structure determination with automated NOE assignment using the new software CANDID and the torsion angle dynamics algorithm DYANA**. Torsten Herrmann, Peter Güntert, and Kurt Wüthrich (2002). J. Mol. Biol. *319*, 209–227.

Combined automated NOE assignment and structure determination module (CANDID) is a new software for efficient NMR structure determination of proteins by automated assignment of the NOESY spectra. CANDID uses an iterative approach with multiple cycles of NOE cross-peak assignment and protein structure calculation using the fast DYANA torsion angle dynamics algorithm, so that the result from each CANDID cycle consists of exhaustive, possibly ambiguous NOE cross-peak assignments in all available spectra and a three-dimensional protein structure represented by a bundle of conformers.

□ **Crystal structure of aspartate racemase from *Pyrococcus horikoshii* OT3 and its implications for molecular mechanism of PLP-independent racemization**. Lijun Liu, Kousuke Iwata, Akiko Kita, Yutaka Kawarabayasi, Masafumi Yohda, and Kunio Miki (2002). J. Mol. Biol. *319*, 479–489.

There exists a d-enantiomer of aspartic acid in lactic acid bacteria and several hyperthermophilic archaea, which is biosynthesized from the l-enantiomer by aspartate racemase. The crystal structure of aspartate racemase from the hyperthermophilic archaeum *Pyrococcus horikoshii* is the first structure reported reveals that this enzyme forms a stable dimeric structure with a strong three-layered inter-subunit interaction, and that its subunit consists of two structurally homologous α/β domains, each containing a four-stranded parallel β-sheet flanked by six β-helices. Two strictly conserved cysteine residues (Cys82 and Cys194), which have been shown biochemically to act as catalytic acid and base, are located on both sides of a cleft between the two domains. The spatial arrangement of these two cysteine residues supports the "two-base" mechanism but disproves the previous hypothesis that the active site of aspartate racemase is located at the dimeric interface. The structure revealed a unique pseudo mirror-symmetry in the spatial arrangement of the residues around the active site, which may explain the molecular recognition mechanism of the mirror-symmetric aspartate enantiomers by the non-mirror-symmetric aspartate racemase.

□ **The structure of the mammalian 20S proteasome at 2.**75 Å resolution. Masaki Unno, Tsunehiro Mizushima, Yukio Morimoto, Yoshikazu Tomisugi, Keiji Tanaka, Noritake Yasuoka, and Tomitake Tsukihara (2002). Structure *10*, 609–618.

The 20S proteasome is the catalytic portion of the 26S proteasome. Constitutively expressed mammalian 20S proteasomes have three active subunits, 1, 2, and 5, which are replaced in the immunoproteasome by interferon-inducible subunits 1i, 2i, and 5i, respectively. The crystal structure of the bovine 20S proteasome was determined at 2.75 Å resolution. The structures of 2, 1, 5, 6, and 7 subunits of the bovine enzyme were different from the yeast enzyme but enabled the bovine proteasome to accommodate either the constitutive or the inducible subunits. A novel N-terminal nucleophile hydrolase activity was proposed for the 7 subunit. The authors also determined the site of the nuclear localization signals in the molecule. A model of the immunoproteasome was predicted from this constitutive structure.

□ **Tandem DNA recognition by PhoB, a two-component signal transduction transcriptional activator**. Alexandre G. Blanco, Maria Sola, F. Xavier Gomis-Rüth, and Miquel Coll (2002). Structure *10*, 701–713.

PhoB is a signal transduction response regulator that activates nearly 40 genes in phosphate depletion conditions in *E. coli* and closely related bacteria. The structure of the PhoB effector domain in complex with its target DNA sequence, or *pho* box, reveals a novel tandem arrangement in which several monomers bind head to tail to successive 11-base pair direct-repeat sequences, coating one face of a smoothly bent double helix. The protein has a winged helix fold in which the DNA recognition elements comprise helix 3, penetrating the major groove, and a hairpin wing interacting with a compressed minor groove via Arg219, tightly sandwiched between the DNA sugar backbones. The transactivation loops protrude laterally in an appropriate orientation to interact with the RNA polymerase 70 subunit, which triggers transcription initiation.

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